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INCORPORATION OF URTICATING HAIRS INTO SILK: A NOVEL DEFENSE MECHANISM IN TWO NEOTROPICAL TARANTULAS (ARANEAE, THERAPHOSIDAE)

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ABSTRACT

Two species of New World theraphosid; *Theraphosa leblondi* from French Guiana, and *Megaphobema* sp. from Ecuador incorporate abdominal setae into silk constructs. *Theraphosa* incorporates setae into the egg sacs and the silk mats on which they molt. *Megaphobema* sp. includes them in the egg sacs only. The setae used in the egg sacs by both these spiders are from the lateral region of the abdomens, the setae which *Theraphosa* uses in the silk mat are from the lateral and posterior regions. All abdominal regions tested on *Theraphosa* had urticating hairs present. To test the possible benefits of this behavior, the egg sacs and silk mats were tested for urticarial effect. The egg sacs failed to elicit any urticarial response in either humans or two species of mouse (*Mus musculus* and *Peromyscus* sp.). Egg sac material with or without setae was found to be an effective barrier to the larvae of the fly *Megaselia scalaris*. The silk mats of *T. leblondi* were found to be more effective at stopping the movement of *M. scalaris* larvae than theraphosid silk which lacked them.

INTRODUCTION

Urticating setae have been well documented in both the larval and adult instars of lepidopterous insects, particularly those in the family Saturniidae. The urticating setae of the Lepidoptera are known to employ either a chemical urticant, mechanical irritation, or both (Goldman et al 1960; Pesce and Delgado 1971). Mygalomorph spiders in the family Theraphosidae have also been known to possess urticarial setae (Bates 1836), but only recently has the phenomenon been examined (Cooke et al. 1972; Cooke et al. 1973).

Cooke et al. (1972) examined specimens in museum collections and described four basic urticating hair types in New World theraphosids (Old World tarantulas apparently lack them). In contrast to the urticating setae of the Lepidoptera, the urticating hairs of tarantulas rely on mechanical irritation alone. These setae are characterized by a penetrating end (which may be at the proximal or distal end), with fine barbs located along the point and longer barbs along the shaft. The base of the hair has a constriction which serves as a break-off point. Tarantula

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defensive hairs are concentrated on the posterior region of the abdomen, although there is an exception to this in the genus *Ephebopus* (Marshall and Uetz 1990). Most tarantulas possess a suite of behaviors which accompany defensive hair shedding. These may be stridulating, rearing and striking with the first two pairs of legs, and attempting to bite. The hairs are shed by rapid downward strokes of one or both of the fourth legs with the ventral surface of the tibia being applied to the posterior abdomen.

Two tarantula species; *Theraphosa leblondi* Latreille (1804) from French Guiana, and *Megaphobema* sp. Pocock (1901) from Ecuador (adult male and female specimens have been deposited in the collection of the Queensland Museum, Brisbane, Australia) have been observed to incorporate lateral abdominal setae into their egg sacs. Additionally, captive *Theraphosa* have been observed to shed hairs onto the silk mat upon which they molt. The phenomenon of incorporating urticating hairs into silk constructs has also been noted for *Avicularia* sp. in Trinidad, which apparently include the hairs in their retreats for defense against predators (A. Bordes in Cooke et al. 1972). In this study we investigate the defensive use of urticarial hairs by *Theraphosa*; their distribution on the abdomen, and their incorporation into shedding mats and egg sacs.

METHODS

Specimens of *Theraphosa* and their egg sacs were collected in the field in French Guiana between 1981 and 1988, and egg sacs were also collected from the laboratory colony. The specimens of *Megaphobema* were collected in the vicinity of Puerto Misuali, Ecuador in December of 1984. The shedding mats were collected from the cages of recently molted *Theraphosa* and stored for later use. *Megaphobema* has not been observed to make such mats.

To investigate the range of distribution of urticating hairs on the abdomen of *Theraphosa*, a comparative survey of hairs on three regions (lateral, dorsal, and posterior) of the abdomens of five preserved specimens was made. The lateral area was chosen as it was the region from which the hairs were shed for the egg sac, the dorsal area because it is a region not known to be associated with any hair shedding behavior, and the posterior area as it is the site of the hairs used in defense. A 1.0 mm square sample of cuticle was dissected out of each site from each specimen and the hairs were scraped off onto a microscope slide and dispersed in a drop of mounting medium by stirring with a probe. Four regularly spaces, parallel transects were taken across the slide and all the hairs were counted and classified as urticating or non-urticating.

The pubescence of the egg sacs of both *Theraphosa* and *Megaphobema* is obvious to the unaided eye. Scanning electron micrographs were prepared of *Theraphosa* egg sac material for closer examination of the structure of the silk-hair matrix. To examine the composition of hairs in the egg sac material, 1.0 mm square samples were taken from five egg sacs. The silk-hair matrix was teased apart, mounted on a slide and the two hair types counted in total. The inclusion of hairs into the shedding mats was measured by taking a 2.0 mm square sample from five shedding mats produced by captives. The material was shredded and dispersed in mounting medium as with the egg sacs. The hairs were counted and classified in total.

To test the urticarial action of the egg sac material against predators, studies were conducted on three vertebrate species, and one invertebrate species. The effect of the egg sac material on humans was tested by rubbing an egg sac against the underside of the forearms of three human volunteers. This was seen as adequate, since the human response to the defensive shedding of posterior abdominal hair by *Theraphosa* is immediate and strong. The shed hairs are borne up on air currents, resulting in a burning, itching sensation on exposed skin surfaces and in the upper respiratory tract. In a test of the egg sac material's effect on a small vertebrate predator model, six wild-caught deer mice (*Peromyscus* sp.) were used. A sample of egg sac material was applied to the mouths of restrained individuals by holding a piece in a forceps and rubbing it around the mouth-nose area, after which the mouse was returned to its cage and observed for fifteen minutes. In both these tests, any inflammation or behavioral evidence of itching was considered a positive response.

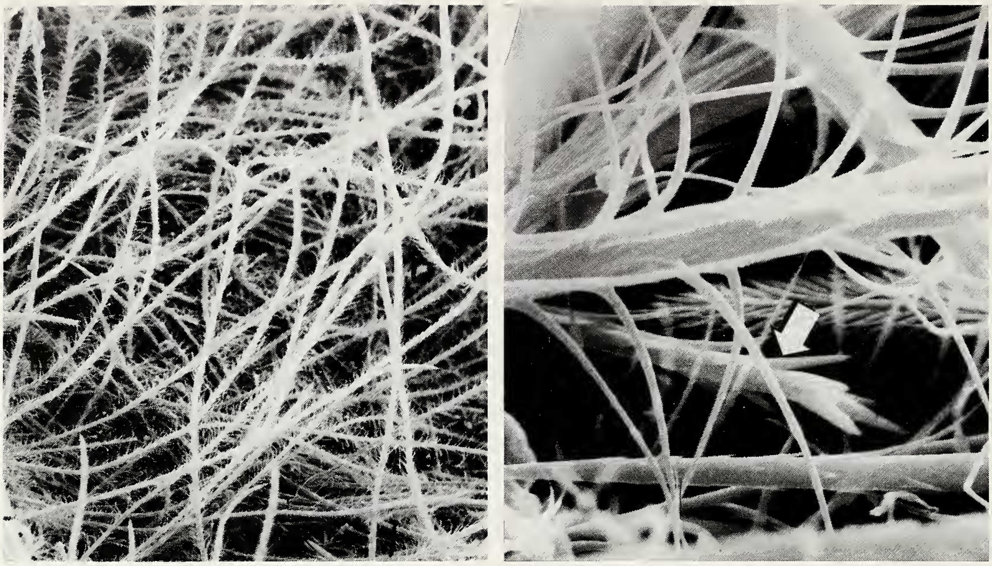
To test for the effect of ingesting hairs, a 1.0 cm square sample of egg sac material was shredded and incorporated into 30.0 gm of peanut butter and offered to the deer mice. A second test was performed to examine the effectiveness of intact egg sac material in deterring vertebrate predation. Samples of material from *Theraphosa* egg sacs were used to enclose the ends of two 10.0 cm by 3.5 cm cylindrical plastic vials that were baited with peanut butter. White laboratory mice (*Mus musculus*) were used. The mice were tested in two groups of five. First, they were fed peanut butter to accustom them to the smell and taste, and then they were fasted for six hours, having free access to water, after which they were offered the tubes (one to a group).

A phorid fly occurs in association with *Theraphosa* in French Guiana (Marshall, pers. obs.). This species belongs to the genus *Megaselia*, and is undescribed (W. H. Robinson pers. comm.). Adult flies have been observed on the spiders in both the field and captivity; the late instar larvae are seen on the cephalothorax and femora, and puparia were found affixed to the cepahlothoracic apodeme and the femora. As this fly is the only known parasite of *Theraphosa* (other than an unidentified mite) a congeneric phorid (*Megaselia scalaris*) was selected to test the deterrent effect of the silk-hair constructs. The flies were trapped from a laboratory cricket colony. *M. scalaris* is a common scavenger, and freely oviposits on dead animal material. Material from two field collected *Theraphosa* and one captive-produced *Brachypelma smithii* Simon (1891) egg sac was used to enclose the ends of patent-lip vials baited with dead crickets. *Brachypelma* was used as it does not include hairs in the egg sac. One vial capped with fine nylon mesh was used as a control. These seven vials were placed in a cage with approximately 60 flies.

The shedding mats were also tested using larval *Megaselia scalaris*. The ability of these larvae to move about on the shedding mats was compared to the total distance travelled on non-pubescent silk matting laid down by captive *Avicularia* sp. from French Guiana (this silk had been examined for setae and none were found). The trials were run for 10 minutes.

RESULTS

Both field-collected and captive-produced *Theraphosa* egg sacs are pubescent in



Figures 1, 2.—SEM of *Theraphosa* egg sac material: 1, the outer covering of hair; 2, a close-up with a Type III urticating hair indicated by the arrow.

appearance. The scanning electron micrographs revealed that the egg sac material is covered with a mixture of the long, non-urticarious body hairs and the smaller urticarious hairs (Figs. 1, 2). The much larger non-urticarious hairs are the most obvious, despite the numerical dominance of the urticating hairs. In the process of making the egg sac, the female *Megaphobema* and *Theraphosa* denude the lateral regions of their abdomen (Figs. 3, 4). This behavior is in contrast to the shedding of posterior abdominal hairs during defensive displays. While *Megaphobema* egg sacs were not microscopically examined, a captive specimen of *Megaphobema* was observed in the process of producing an egg sac. The female begin by laying down a circular mat of silk within the retreat by standing in the center and turning around. The spider would then pause and shed the hairs with slow, downward stroking motions of the fourth tarsal scopula against the lateral areas of the abdomen. Alternate sides were used between bouts of hair shedding. The behavior is similar to preening in both tempo and use of the tarsal scopulae.

Figures 3, 4.—*Megaphobema* sp. females before (3), and after (4) production of an egg sac. The denuded lateral regions of the abdomen are visible in 4, indicated by an arrow.

Table 1.—Two-way ANOVA on the proportion of urticating hairs on the dorsal, lateral, and posterior abdominal regions of five females of *Theraphosa leblondi.*

	SS	*df*	*MS*	*F* ratio	*P*
Regions	1669.91	2	834.96	25.47	$P << 0.005$
Specimens	296.62	4	74.16	2.62	$P > 0.1$
Error	262.24	8	32.78		
Total	2228.77				

While egg-laying behavior has never been observed in *Theraphosa*, it is assumed to be the same.

In the samples taken from *Theraphosa* abdomens, urticating setae were found at all sites sampled, and were the numercially dominant type. The percent urticating hairs among the setae on the dorsal region of the abdomen was 87.0 ± 3.0 (mean ± one standard deviation); the lateral 74.0 ± 0.06; and the posterior 95.0 ± 7.0. The urticating hairs were all what Cooke et al. (1972) refer to as type III (V. Roth, pers. comm.). The urticating hairs from the posterior abdomen were longer, ranging from 0.5-1.0 mm. In the other two sites sampled, the hairs were approximately 0.1 mm.

A two-way ANOVA testing variation between sites and between spiders was performed using the arc-sine transformed proportion of urticating hairs. The difference between the sites was significant, but not between spiders (Table 1). When the proportion of urticarial hairs in the egg sac material and the lateral abdomen were compared using a Mann-Whitney *U* test, the results were not significantly different. The five egg sacs had 66.0 ± 2.0 percent urticating hairs. In the five shedding mats both the long posterior and short lateral urticating hairs were mixed, and together comprised 86.0 ± 4.0 of the total hairs. Taken separately, the long urticating hairs constituted 24.0 ± 14.0, and the short urticating hairs were 63.0 ± 17.0.

In the tests for an urticarial response to egg sacs applied to the skin, no itching was reported by the human volunteers. Mice did not appear distressed nor did they indulge in excessive grooming behavior after similar exposure.

In the first feeding test, all the peanut butter-egg sac material mixture was consumed. Microscopic examination of the feces revealed both urticating and non-urticating hairs had been ingested and passed through, and the mice appeared normal. In the second feeding test, using intact material, the results were similar. The mice initially investigated the tubes, sniffing and nibbling at the material, and then ignored them. The tubes were left in the cages overnight, and 15 hours later, the egg sac material had been chewed and partially consumed, along with a portion of the peanut butter. There were no egg sac fragments in the cage, and it was mostly gone from the tubes. Examination of the feces once again revealed that the hairs (and silk) had been ingested and passed through without adverse effects.

The phorid experiment was terminated after 72 hours when the adult flies were dead. The flies had oviposited on the control vial (with the mesh on top), one *Brachypelma* vial and one *Theraphosa* vial. Larvae were observed in the control vial only. The *Theraphosa* shedding mats were more effective at slowing the progress of the phorid larvae (mean distance in mm travelled in 10 minutes ± one standard deviation: *Theraphosa* mats, 8.8 ± 6.8; *Avicularia* webbing, 42.0 ± 33.5;

$t = 2.17$, $df = 8$, $p = 0.06$). All the phorid larvae on the *Theraphosa* webbing eventually stopped. On the *Avicularia* webbing, three stopped, one left the webbing, and one continued moving for the duration of the trial. The greater variability of the distance travelled by the control group resulted in a greater sample variance, which is responsible for the marginal significance value. Examination with a dissecting microscope revealed that on *Avicularia* silk the setae of the larval flies which stopped had become entangled in the loose silk strands. The phorid larvae on the *Theraphosa* shedding mats were likewise observed under magnification and seen writhing around, coated with *Theraphosa* hair, having anchored themselves with their posterior appendages.

DISCUSSION

The tarantulas of the New World have evolved a unique defensive strategy utilizing urticating setae, which is a characteristic shared only with the Lepidoptera. The variety of hair types and apparent uses indicates the utility of such an adaptation. Why it is only found in the New World theraphosid fauna, however, remains a mystery.

No egg sac predators of *Theraphosa* have ever been recorded. As this is a little-studied species, this does not preclude their existence. It is obvious that both *Theraphosa* and *Megaphobema* are making an investment in both time and energy, as well as in paying the possible costs that shedding a complete coating of hairs may confer (i.e., loss of boundary layer effects, parasite defense).

Until we know more about the predators and parasites of *Theraphosa* there may be no way to know what selective forces induced the evolution of the unique behaviors leading to the inclusion of setae in egg sacs. However, evidence from experimental studies reported here allows some speculation about possible selective agents.

The vertebrate tests indicate that the silk-hair matrix has no negative effect on three mammalian species (although these species have no previous ecological or evolutionary exposure to theraphosid spiders). In its egg sacs *Theraphosa* uses a field of hairs that contains the lowest proportion of urticating hairs, and a hair type that is distinct from those used in individual defense against vertebrates. These findings argue against a hypothesis that this defensive mechanism is adapted to deter vertebrate predators. Additionally, during incubation, both *Theraphosa* and *Megaphobema* guard their egg sacs constantly and with vigor. This behavioral investment may be considerable, as *Theraphosa* in captivity attend the egg sac for 11 weeks until hatching (Marshall pers. obs.). As female *Theraphosa* will engage in typical defensive displays while holding the egg sac in their fangs, it seems likely that an attack by a large, vertebrate egg sac predator would be warded off at an early stage in the predatory sequence, or the female herself would be the target of attack. The tests with *Megaselia scalaris* larvae indicate that egg sac material with or without setae may be an effective barrier to penetration by larval parasitoids or scavengers. However, the behavior of *M. scalaris* larvae on the *Theraphosa* shedding mats indicates that the incorporated hairs function as a means of deterring parasites. During the molting process a spider is clearly more vulnerable to boarding (or re-boarding) by ectoparasites. On at least one occasion, a captive *Theraphosa* was seen to have a later instar

Megaselia sp. larvae moving about on one of its patellae as the spider prepared to molt. It is also noteworthy that while both *Theraphosa* and *Megaphobema* include setae in the egg sacs, only the species known to have the phorid ectoparasite spins a shedding mat which includes setae. This adds credence to the hypothesis that combining urticating hairs with silk is an adaptation against larval dipteran parasitoids.

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